

AMENDMENTS TO THE CLAIMS:

This listing of the claims will replace all prior versions, and listings, of claims in the present application.

LISTING OF THE CLAIMS:

31. (Currently amended) A method of isolating at least one anti-ligand to at least one target ligand comprising the steps of:

(i) providing a library of anti-ligands;

(ii) providing an amount of a first subtractor ligand a first population of ligands comprising a ligand fixed to or incorporated in a subtractor ligand construct;

(iii) providing an amount of a second target ligand a second population of ligands comprising the same ligand as step (ii), fixed to or incorporated in a target ligand construct;

(iv) determining amounts of first subtractor ligand construct and second target ligands ligand construct using one or more equations derived from the universal law of mass

$$\text{action } \frac{[C]^c[d]^d}{[A]^a[B]^b} = K_{eq},$$

where:

A, B, C & D = are the participants in the reaction (reactants and products)

a, b, c, & d = the coefficients necessary for a balanced chemical equation so as to permit isolation of at least one anti-ligand to at least one target ligand;

(v) providing the amount of the first subtractor ligand construct determined in step (iv);

(vi) providing the amount of the second target ligand construct determined in step (iv);

(vii) providing separation means for isolating anti-ligand bound to the second target ligand construct from anti-ligand bound to the first subtractor ligand construct, wherein said separation means for the subtractor and target ligand constructs can be either the same or different;

(viii) exposing the library of (i) to the ligands of (v) and (vi) to permit binding of anti-ligands to ligands; and

(ix) isolating the anti-ligand bound to the second ligand fixed to or incorporated in the target ligand construct with using the separation means.

Claim 32-33 (Canceled)

34. (Currently amended) A method as claimed in ~~one of claims~~ ~~claim~~ 31, 32 or 33 comprising a further step of releasing the anti-ligand from the ~~second~~ target ligand.

35. (Currently amended) A method as claimed in claim 31 or 32 whereby steps (ii) to (ix) are repeated one or more times.

36. (Currently amended) A method as claimed in claim 31 or 32 wherein the amount of one of the ~~first~~ subtractor ligand construct or ~~second~~ target ligand construct is provided in excess of the amount of the other of the ~~first~~ subtractor ligand construct or ~~second~~ the target ligand construct.

37. (Previously presented) A method as claimed in claim 36 where the excess of ligand is of 10 to 100 fold.

38. (Currently amended) A method as claimed in ~~claims~~ ~~claim~~ 31 or 32 wherein the equation of (iv) is

$$bA = \frac{(A + T + (K_d)x(CxV))}{2} - \sqrt{\frac{(A + T + (K_d)x(CxV))^2}{4} - AxT}$$

where

bA = Bound anti-ligand

A = Total number of anti-ligand

T = Total number of ligands

C = Avogadro's constant ( $6.022 \times 10^{23}$  particles/mole)

V = Reaction volume (litres)

$K_d$  = Equilibrium dissociation constant.

39. (Currently amended) A method as claimed in claims 31 or 32 wherein the equation of (iv) is:

$$bA = \left\{ \frac{(A + T + (K_d)x(CxV))}{2} - \sqrt{\frac{(A + T + (K_d)x(CxV))^2}{4} - AxT} \right\} x \left\{ \frac{(T_p x C_p)}{(T_p x C_p) + (T_s x C_s)} \right\}$$

where

$bA_p$  = Bound anti-ligand

$T_p$  = The number of ligands on  $C_p$

$T_s$  = The number of ligands on  $C_s$

$C_p$  = The number of target ligand constructs

$C_s$  = The number of subtractor ligand constructs

$A$  = Total number of anti-ligand

$T$  = Total number of ligands

$C$  = Avogadro's constant ( $6.022 \times 10^{23}$  particles/mole)

$V$  = Reaction volume (litres)

$K_d$  = Equilibrium dissociation constant.

40. (Currently amended) A method as claimed in claim 31 or 32 wherein the separation means are selected from at least one of a solid support, cell membrane and/or portions thereof, synthetic membrane, beads, chemical tags and free ligand.

41. (Previously presented) A method as claimed in claim 40 whereby the separation means are cell membranes and/or portions thereof.

42. (Currently amended) A method as claimed in claim 41 whereby the ~~first~~ subtractor and ~~second~~ target ligands are fixed to and/or incorporated within separate cell membranes and/or portions thereof.

43. (Currently amended) A method as claimed in claims ~~claim~~ 31 or 32 whereby the separation means of the ~~first~~-subtractor and ~~second~~ target ligand constructs have a different density.

44. (Currently amended) A method as claimed in claim 43 wherein the separation means

of the first subtractor ligand construct is of a lower density than the separation means of the second target ligand construct.

45. (Currently amended) A method as claimed in claim 44 wherein the separation means of the first subtractor ligand construct is a membrane vesicle.

46. (Currently amended) A method as claimed in claim 44 wherein the separation means of the second target ligand construct is a whole cell membrane.

47. (Currently amended) A method as claimed in claim 31 or 32 whereby the isolation of anti-ligand bound to second target ligand step (ix) is performed by at least one of density centrifugation, solid support sequestration, magnetic bead sequestration, chemical tag binding and aqueous phase partitioning.

48. (Previously presented) A method as claimed in claim 47 whereby the isolation step is performed by density centrifugation.

49. (Previously presented) A method as claimed in claim 48 wherein the density centrifugation is performed using a sucrose-polymer gradient.

50. (Currently amended) A method as claimed in claim 31 or 32 wherein the library of step (i) is a display library comprising a plurality of library members which display anti-ligands.

51. (Previously presented) A method as claimed in claim 50 wherein the library is a phage display library.

52. (Currently amended) A method as claimed in claim 31 or 32 wherein the subtractor and target ligands are independently at least one from antigens; receptor ligands; and enzyme targets that comprise at least one selected from carbohydrate; protein; peptide; lipid; polynucleotide; inorganic molecules and conjugated molecules.

53. (Currently amended) A method as claimed in claim 31 or 32 wherein the library of anti-ligands is composed of at least one selected from antibodies, and antigen binding variants, derivatives or fragments thereof; scaffold molecules with engineered variable surfaces; receptors; and enzymes.

54. (Currently amended) A method as claimed in claim 31 or 32 comprising a further step of exposing the ligand and its separation means to a stimulus which influences the expression of target ligands on said ligand constructs.

Claims 55-59 (Canceled)